Guiding Principles

1. The reagents core should serve the entire community.
(It is possible to serve the community and to advance diagnostics.)

2. Validation, annotation, coordination, and curation into a database are the missing links, and the most valuable service the Reagents Core can provide.

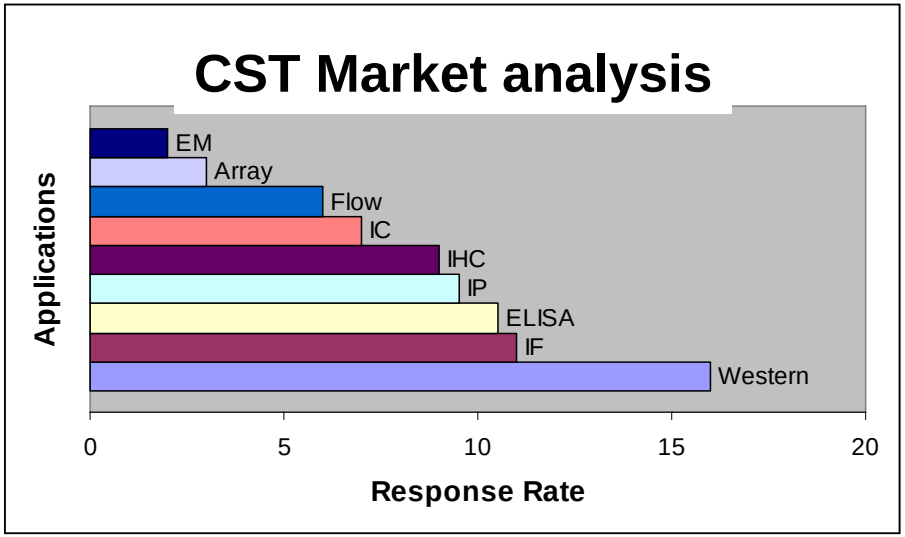
3. New, higher-throughput technologies should be developed in preparation for expanding this project to target the entire proteome.

Sources	Applications	Validation/Annotation
commercially available		
unscreened hybridomas	X	
requests from community		
antibodies funded by grants		

In what types of applications should affinity reagents be required to perform?

Research Areas of Antibody Customers

- 56% on cellular development
- 47% on gene/protein regulation
- 44% on molecular mechanisms of human diseases
- 39% on molecular structure/function characterization
- 28% on non-human disease



Shared by Epitomics

- 79% Western
- 60% ELISA
- 55% Immunofluorescence
- 50% IP
- 44% IHC

Shared by Neoclone

Survey of most recent 100 projects

- 58% Western
- 34% ELISA
- 28% IHC
- 9% Immunoaffinity Purification
- 6% IP
- 6% FACS
- 2% Chromatin IP (ChIP)

Shared by Abnova

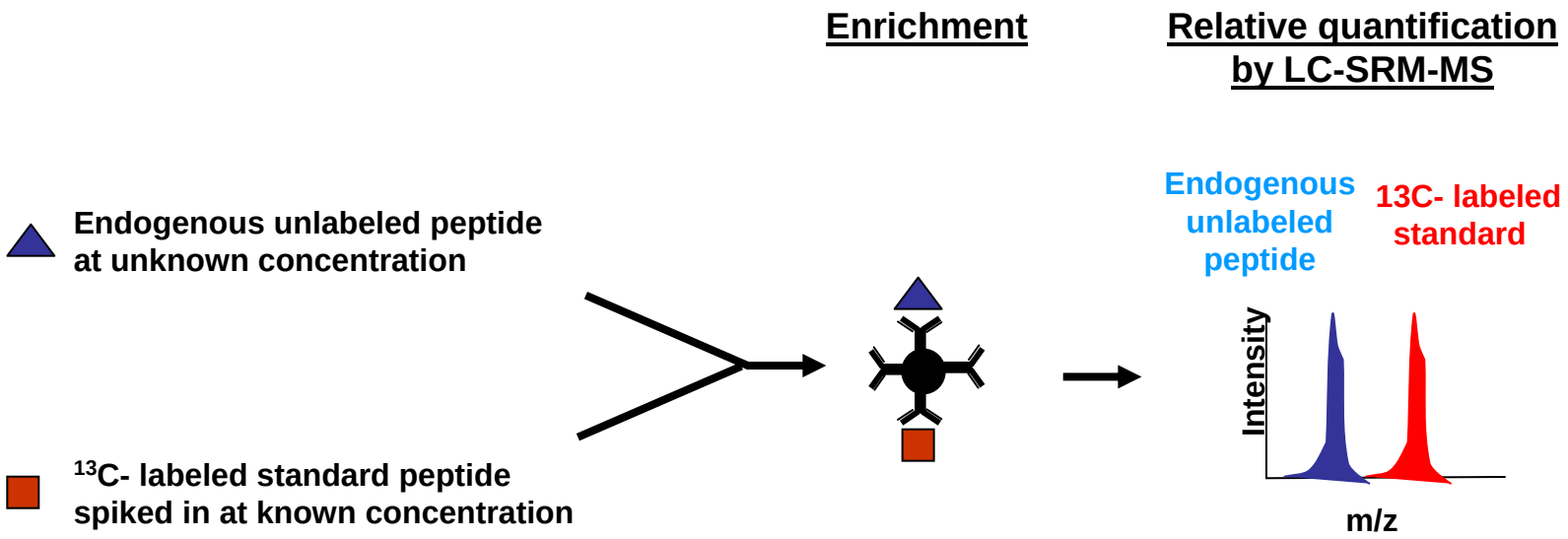
- WB
- IHC
- IP
- ELISA

Antibodies by Design, a Division of MorphoSys

- Westerns
- IHC
- ELISA
- FACS
- IP

SISCAPA: an emerging technology.

Journal of Proteome Research 2004, 3, 235-244



>10^4 fold enrichment S2N

Recommended Target Applications

Sources	Applications	Validation/Annotation
commercially available	1. **Western** 2. **ELISA** 3. **Immunofluorescence / FACS** 4. **Immunoprecipitation** 5. **Immunohistochemistry** 6. **SISCAPA**	application-independent
requests from community		application-dependent
unscreened hybridomas		

Priority Questions

1. How sensitive is the antibody (affinity/avidity)?

    - Biacore?
    - Antigen spike experiments?

2. Does my antibody bind what I want it to bind?

    - tissue arrays (+/- controls)
    - protein or prEST arrays
    - cell lines +/- expression of target
        - siRNA
        - cDNA
        - KO

3. How specific is my antibody? (S2N)

    - tissue arrays (+/- controls)
    - protein or prEST arrays
    - cell lines (+/- controls)
    - X-SPECIES??

Validation methods for antibodies (application-independent)

Method	Description	Examples
Antigen-based	Assays based on the antigen used for immunization (immunogen)	ELISA, protein arrays, SPR, antigen adsorption
Target-based	Analysis of native or partially denaturated protein from natural sources (such as cell lysates)	Western blots, IHC, immunocapture (Ñpull-downsÓ)
RNA-based	Comparison of expression levels at the protein and RNA level	Transcript profiling, in situ hybridizations
DNA-based	Bioinformatic analysis using predictive algorithms (as compared to experimental data)	Signal peptide, transmembrane regions, localization signals
Genetics-based	The use of genetic mutants or recombinant constructions to validate the target	Transgenetics, RNAi, GFP-fusions (subcellular localization)
Epitope-based	Comparison of two of more antibodies directed to different parts of the same target	Antibodies to PrESTs or synthetic peptides

Uhlen et al (2005) A human protein atlas for normal and cancer tissues, Mol Cell Proteomics, in press (on-line)

Application-independent

1. What are the binding affinities of the Ab? Include Biacore measurements with each Ab.
2. Characterize specificity by screening on arrays of expressed human proteins (prESTs?), reporting prevalence of cross-reactions.
3. Validate target identity with control lysates (siRNA?).
4. Better information on species cross-reactivity (human, mouse, rat, etc)
5. Include specific antigen information/sequence
6. Formulation of Abs (carriers and buffers included in mix)
7. Accurate shelf life and storage conditions
8. Provide both Ab concentration (mg/ml) and working dilutions (1:1000 etc.)
9. Showing enough data from reagents is always an issue. If groups could share more data with the companies without publication issues, then perhaps it could benefit everyone using the reagents.
10. Test the new lots in all the applications side by side with the old lot.

Application-Specific Validation

1. Western blot: show entire blot with positive and negative controls, show background signal, results on multiple cell lines, cross-species "zoo blots"

2. ELISA: standard curve showing full dynamic range (upper and lower limits of detection) and c.v. of analyte spiked into plasma/cell lysate.

3. Immunofluorescence: confocal images from multiple cell lines and tissues, including positive and negative controls where possible.

4. Immunoprecipitation: western blots of both initial lysate and captured proteins, show WB data for protein complex pulldowns

5. SISCAPA: MS-based determination of enrichment, specificity, and limit of quantification of target peptide relative to isotopically labeled peptide spiked into complex mixture (plasma or cell lysate).

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue specimens afford the best morphology and represent the broadest possible range of tissues and diseases.

•Screen tissue arrays and cell line array (positive and negative control cell lines in paraffin blocks)

•For ligands that have been studied previously, the pattern and anatomic distribution of staining should be consistent with what has been reported in the literature. (immunohistochemistry, Northern-blot analysis, in situ hybridization, EST expression profiling, radioimmunoassay, and ligand-binding data)

•For “orphan ligands,” supporting data are not available, and evaluation of the specificity and validity of IHC findings relies more heavily on cross-concordant findings with multiple antibodies and on gene expression studies such as Northern-blot analysis or in situ hybridization.

•Another criterion for evaluating antibody specificity and performance is that the subcellular localization of the staining signal should reflect the protein's function.